The Effects of Visible Light Radiation (400–500 nm) on Enzymatic Activity of Collagenase

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Abstract— Debridement is the process of removing necrotic burden or contaminated tissue from a wound bed until surrounding healthy tissue is exposed. Enzymatic debridement helps to remove non-viable tissue which can otherwise delay wound healing and lead to infection [1]. Collagenase enzyme is known to be able to promote cellular responses to injury and wound healing *in vivo*. Collagenase shows more selectively on denatured collagen in devitalized tissue. This selectivity is beneficial as it keeps the vital tissue and growth factors crucial to wound healing intact. There are studies which have shown that applied electromagnetic radiation (EMR) in the visible light range can modulate protein and cellular activity. Here we validate experimentally the hypothesis of the Resonant Recognition Model (RRM) that selectivity of protein activities is based on specific resonant electromagnetic interactions [2]. The computational analysis of 28 collagenase sequences was performed and the activation frequency/wavelength range was determined to be 450–460 nm. To evaluate this range, the Collagenase enzyme solutions were irradiated by monochromatic light of 400 to 500 nm. The kinetics of the chemical reaction was measured by continuous monitoring of the changes in absorbance of collagen at 570 nm. The results revealed that collagenase activity can be modulated at the particular wavelengths of 450 nm, 456 nm, and 460 nm, which is within the activation wavelength range defined computationally. This finding indicates that enzyme function can be modified by an applied electromagnetic radiation of defined frequency, which may contribute to the development of a new clinical therapy for wound healing promotion.

1. INTRODUCTION

Debridement is an essential treatment process used in wound bed preparation. Since devitalized tissue can obstruct healing of a wound, debridement offers a comprehensive approach to removing barriers to healing and creating a wound environment for healing promotion and thus, reducing risk of local infection [1]. There are a number of methods currently used for debridement of wounds. These include surgical, chemical, enzymatic, mechanical and biological techniques [5, 6]. Although enzymatic debridement is a time consuming process, it is still a primary technique for wound debridement in certain cases, especially when alternative methods such as surgical or conservative sharp wound debridement are not feasible due to bleeding disorders or other complication [5]. Of particular interest to this study are the enzymatic debridement method and the possibility of accelerating/promoting this process aiming at its enhanced clinical practice in wound management.

There is evidence that applied EMR in the visible light range can modulate protein and cellular activity [7, 8]. Reported studies show that endochondral bone formation can be regulated by exogenously applied biophysical stimuli that include EMR. Some studies revealed that exposures to pulsed electromagnetic fields (PEMF) enhance chondrogenic differentiation and the synthesis of cartilage extracellular matrix proteins. Studies focused on investigating the effects of visible light on cell proliferation and their metabolisms were reported [9]. Biological processes can be modulated by induction of light of particular frequencies of applied EMR [3, 4]. Our previous studies show that activity of L-Lactate Dehydrogenase can be affected by external light of particular wavelengths, computed using the Resonant Recognition Model [3, 4]. The RRM theory states that an external EMR at a particular frequency would produce resonant effects on protein biological activity [2, 3]. The resonant absorption and resonant interactions have been proposed as an explanation for the marked sensitivity of living systems to EMR [10]. Each biological process involves a number of interactions between proteins and their targets, which are based on energy transfer between the interacting molecules. Protein interactions are highly selective, and this selectivity is defined within a protein's structure. The RRM is designed for analysis of protein (DNA) interactions and their interaction with EMR [2–4]. In our previous work [2], a relationship between the RRM spectra of some protein groups and their interaction with visible light has been established. The RRM concept states that external EMR at a particular activation frequency would produce resonant effects on protein biological activity [3, 4]. This hypothesis is validated experimentally here by irradiating

collagenase enzyme (that plays a crucial role in enzymatic debridement) by visible light in the frequency range of 400–500 nm.

2. MATERIALS AND METHODS

The RRM is a physico-mathematical approach based on digital signal processing [2]. The application of the RRM involves two stages of calculation. The first is the transformation of the amino acid sequence into a numerical sequence. Each amino acid is represented by its Electron-Ion Interaction Potential (EIIP) value which describes the average energy states of all valence electrons in a given amino acid [2]. A unique number can thus represent each amino acid or nucleotide, irrespective of its position in a sequence. Then the numerical series obtained are analyzed by digital signal analysis methods, Fourier and Wavelet transforms, in order to extract information pertinent to the biological function. A multiple cross-spectral function is defined and calculated to obtain the common frequency components from the spectra of a group of proteins. Peak frequencies in such a multiple cross-spectral function denote common frequency components for all sequences analyzed.

It was shown in our previous studies that all protein sequences with a common biological function have a common frequency component in the free energy distribution of electrons along the protein backbone. This characteristic frequency was shown to be related to protein biological function [2–4]. It was also shown that proteins and their targets share a characteristic frequency. Thus, it can be postulated that RRM frequencies characterize not only a general function but also a recognition/interaction between the particular proteins and their target at a distance. Thus, protein interactions can be viewed as a resonant energy transfer between the interacting molecules. This energy can be transferred through oscillations of a physical field, possibly electromagnetic in nature [2]. Since there is evidence that proteins have certain conducting or semi-conducting properties, a charge moving through the protein backbone and passing different energy stages caused by different amino acid side groups can produce sufficient conditions for a specific electromagnetic radiation or absorption [2]. A strong linear correlation exists between the predicted and experimentally determined frequencies corresponding to the absorption of electromagnetic radiation of such proteins [2]. It is inferred that approximate wavelengths in real frequency space can be calculated from the RRM characteristic frequencies for each biologically related group of sequences. These calculations can be used to predict the wavelength of the light irradiation, which might affect the biological activity of exposed proteins. The frequency range predicted for protein interactions is from 10^{13} Hz to 10^{15} Hz. This estimated range includes IR, visible and UV light. These computational predictions were confirmed by comparison of: (i) absorption characteristics of light absorbing proteins and their characteristic RRM frequencies [2]; (ii) frequency selective light effects on cell growth and characteristic RRM frequencies of growth factors [2]; and (iii) activation of enzymes by laser radiation [2].

These results indicate that the specificity of protein interaction is based on a resonant electromagnetic energy transfer at the frequency specific for each interaction. A linear correlation between the absorption spectra of proteins and their RRM spectra with a regression coefficient of $K = 201$ was established. Using RRM postulates, a computationally identified characteristic frequency for a protein functional group can be used to calculate the wavelength of applied irradiation, λ, defined as $\lambda = 201/f_{\mathrm{RRM}}$, which could activate this protein sequence and modify its bioactivity [2, 3]. Here, we utilize this relationship to calculate the frequencies/wavelengths that modulate the bioactivity of the selected enzyme and investigate/evaluate their activation experimentally.

3. EXPERIMENTAL STUDY

3.1. Chemicals

TES free acid, Calcium Chloride with Dihydrate, Collagen Type 1, Collagenase were all obtained from Sigma.

3.2. Equipment

As a source of visible light radiation we used Monochromators SPEX 270M: (Princeton Instruments, Trenton, NJ, USA) 1200 g/mm grating, focal length 270 mm, resolution 0.1 nm at 500 nm, dispersion 3.1 nm/mm, range 400–890 nm, RS232 connection with HP 34001A, controlled by LabView 6.1 (National Instruments). For measurement of absorbance of the analyzed enzyme solutions we used Ocean Optics USB2000 spectrometer coupled with USBISS-UV/VIS, (Ocean Optics, FL, USA) range 190–870 nm, USB-2 connection with Pentium IV (Windows XP). Software automatically monitors and saves the absorption coefficient at 570 nm wavelength every 10 sec.

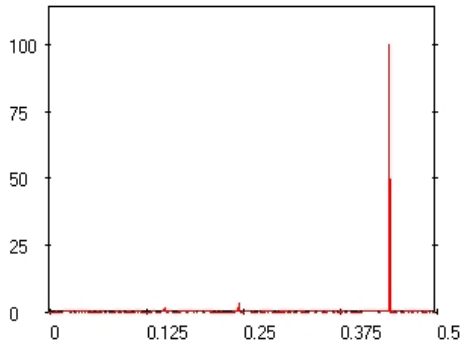

Figure 1: Multiple cross spectral function of 28 vertebrate collagenase sequences. The x axis represents the relative RRM frequency. The y axis represents the normalized intensity.

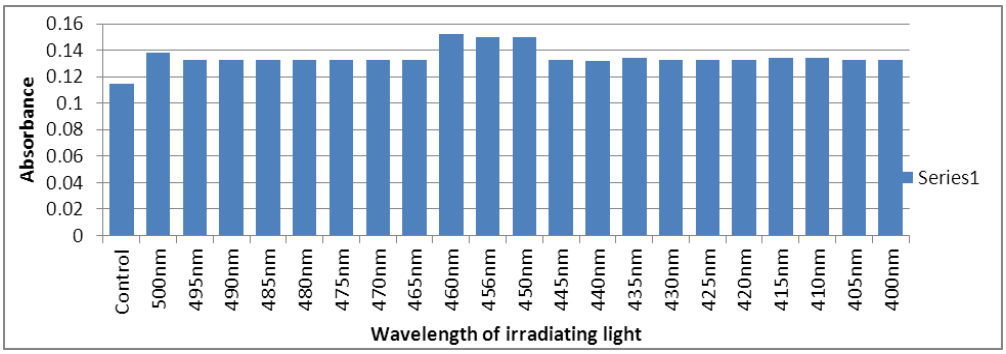

Figure 2: The effect of irradiating light on absorbance of Collagen.

3.3. Enzymatic Assay

Experimental solutions were prepared according to the standard enzymatic assay of collagenase from Sigma-Aldrich. The experiments were divided into two groups: (i) Group 1 (sham-exposure), collagenase sample not exposed to applied irradiation. This sample was used as a control for evaluating effects of irradiation on exposed *vs.* non-exposed samples; and (ii) Group 2, collagenase samples irradiated with light of different wavelengths (400–500 nm, with 5 nm steps) for 600 sec. All experiments were performed at 37°C.

3.4. Experimental Protocol

1. The cuvette, filled with the collagenase solution, was irradiated with light of a particular wavelength (400–500 nm) for 600 sec or incubated for 600 sec for the control enzyme sample;
2. The irradiated or incubated non-exposed samples were added to the already prepared enzymatic substrate solution of Collagen Type I (pH 7.4);
3. The optical density of digested collagen solution is measured at 570 nm (Sigma assay) for each selected irradiating wavelengths (400 nm–500 nm, with a step of 5 nm) and the control sample. With the aim at eliminating the effect of all possible artifacts (PH, temperature, and concentrations), the measurements were repeated three (3) times for each irradiating wavelength to evaluate changes in collagenase absorbance (changes in activity of collagenase solutions) before and after the light exposures.

4. RESULTS

In this study, the RRM was used to compute the RRM characteristic frequency of 28 vertebrate collagenase proteins, that corresponds to their common biological activity. Collagenase primary protein sequences were collected from the NCBI protein database. A multiple cross-spectral analysis was performed resulting in one prominent RRM characteristic frequency identified at $f = 0.4385$ (Fig. 1). This frequency is related to the biological activity of the analyzed Collagenase proteins, as was found in our previous studies.

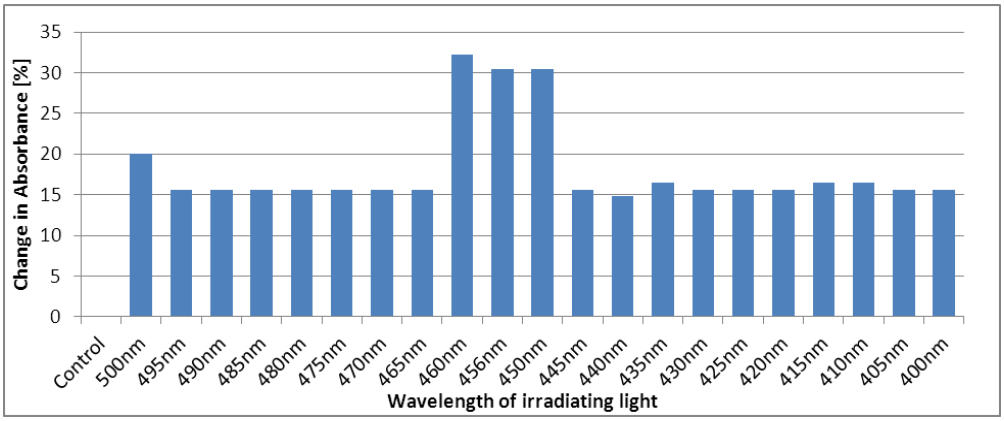

Figure 3: Relative change in Collagen absorbance upon light irradiation, %.

This RRM frequency $f = 0.4385$ is used to calculate the wavelength of external irradiation, λ, which can activate Collagenase protein and modify its bioactivity, $\lambda = 201/f_{\mathrm{RRM}}$. Thus, the wavelength of the EMR required for Collagenase enzyme activation will be at or near 456 nm. To validate this predicted activation wavelength, the experiments were conducted according to the experimental protocol outlined above with the results presented in Fig. 3 and Fig. 4. The results revealed that light (400–500 nm) affects the absorbance of digested collagen (the influence of irradiated collagenase solution) at different degrees.

Figure 2 shows that the selected light exposures increase Collagenase absorbance and thus, affect its enzyme activity. A significant effect (compared to the control sample) is seen at the wavelengths of 450, 456, and 460 nm (Fig. 3) with the increase of 30–32% in Collagen absorbance achieved at these particular wavelengths. The maximum change in absorbance of digested collagen is in the range predicted computationally by the RRM. A change of 20% in absorbance can be seen at 500 nm, and even smaller changes of 15–16% are seen at all other wavelengths (Fig. 3). These findings support the hypothesis that protein activity of Collagenase can be modulated by external light of the particular wavelengths predicted by the RRM approach.

5. CONCLUSIONS

The results presented reinforce the previously developed linear relationship between the calculated RRM frequencies and wavelengths of light radiation. With this correlation in mind, it is now possible to calculate wavelengths of light irradiation which will affect different biological processes. These findings suggest that EMR can be used as a non-invasive treatment to promote enzymatic debridement and thus assist wound healing. The possibility to computationally calculate the RRM frequencies, followed by the use of IR and visible light to produce the desired biological mutations and alterations in proteins will benefit the development of new biomaterials, and advanced technologies.

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